Lasers in Surgery and Medicine 20:332–339 (1997)

Photo-Irradiation Improved Functional Preservation of the Isolated Rat Heart

Qingyan Zhu, MD,[1] Wei Yu, MD,[2] Xiaoping Yang, MD,[1] George L. Hicks, MD,[1] Raymond J. Lanzafame, MD,[2] and Tingchung Wang, PhD[1]*

[1]Department of Surgery, University of Rochester, Rochester, New York 14642
[2]Department of Surgery, Rochester General Hospital, Rochester, NY 14621

Background and Objective: **Photo-irradiation causes a variety of effects in different cells and tissues. We hypothesized that photo-irradiation may improve cardiac preservation based on these observations.**
Study Design/Materials and Methods: **For pre-storage treatment (Pre), the heart in an anesthetized open-chest rat was irradiated using an Argon-dye laser with a wavelength of 660 nm at a fluence of 16.8 J/cm^2 or sham-operated. The heart was excised, perfused with Krebs-Henseleit Buffer, cardioplegically arrested, and stored by immersion at 0°C for 18 hrs. Functional recovery was evaluated by working reperfusion for 30 min. For post-storage treatment (Post), the isolated hearts were stored for 18 hrs at 0°C; laser irradiation at a fluence of 36 J/cm^2 was administered during working reperfusion. Hearts which did not receive irradiation during reperfusion served as control. Furthermore, isolated cardiomyocytes were used to study laser effect on cellular ATP content, catalase activity, and nitric oxide (NO) release.**
Results: **Both Pre and Post groups showed significant improvement in recovery of aortic flow, cardiac output, and work compared to the corresponding control groups ($P<0.05$). Combined Pre/Post laser treatment did not improve function. Investigation using isolated rat cardiomyocytes found that both end-storage ATP and end-reperfusion catalase activity in the laser-treated group were significantly higher than those in the untreated cells ($P<0.05$). NO release increased by 15% in the laser group after 18 hrs of 37°C incubation.**
Conclusion: **Photo-irradiation improves functional recovery of the cold-stored rat heart possibly via conservation of ATP and antioxidant enzyme activity. Lasers Surg. Medicine 20:332–339, 1997.**

Key words: cardiac preservation; photo-irradiation; isolated rat heart

INTRODUCTION

Hypothermic immersion storage of the human heart currently offers 4–6 hrs of storage time thus greatly restricting the donor heart availability for transplantation. Prolongation of storage time is, therefore, necessary to expand the donor organ pool. Various experimental studies have tried to extend the preservation duration. Recently, photo-irradiation (PI) has been demonstrated to be beneficial in various clinical settings such as wound healing [1–5], peptic ulcer [6–8], chronic obstructive lung diseases [9,10], and ischemic myocardial diseases [11–16]. PI promotes cell proliferation and accelerates wound healing [17–20]. Experimentally, PI causes vasodilation [21–23], increases mitochondrial respiration and ATP synthesis [24,25], elevates activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [14,26–28], and en-

*Correspondence to: Dr. Tingchung Wang, Department of Surgery, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642.

Accepted for publication 13 May 1996.

hances nitric oxide (NO) production in rat lymphocytes [29]. However, the potential effects of PI on cardiac preservation have not been explored. Based on these observations, we hypothesized that PI may improve functional preservation of the cardiac explant. In this study, the rat heart was treated with PI before cold-storage, during post-storage reperfusion, or both, and functional recovery was evaluated. Furthermore, isolated cardiomyocytes were used to study laser effect on cellular ATP content, CAT activity, and NO release. We found that either pre- or post-storage laser treatment significantly improved the functional preservation; while combined pre- and post-storage PI did not have any effect. Moreover, pre-storage PI treatment of isolated cardiomyocytes preserved cellular ATP content and CAT activity and promoted NO release providing clues to the possible mechanism(s) of laser irradiation.

MATERIALS AND METHODS

Isolated Rat Heart Perfusion

Storage of the cardiac explant. Male Sprague-Dawley rats (300–350 g) were anesthetized and heparinized as described previously [30]. For pre-storage PI treatment (Pre group), the rat was artificially ventilated via a tracheal cannula at 40 strokes/min with 2.5 ml air/stroke (Harvard Apparatus Rodent Respirator). After the chest was opened, the heart received PI treatment and then was excised. Open-chest control animals (OC) were sham-operated for the same duration but without PI. For post-storage PI treatment (Post group) and its untreated control (closed-chest control, CC), the animal was not ventilated and the heart was excised immediately after the chest was opened; PI was given during reperfusion. For all groups, the excised heart was arrested by immediate immersion in ice-cold Krebs-Henseleit Buffer (KHB), perfused with 36.5°C oxygenated KHB for 9 min, flushed with warm oxygenated CP-11EB (a cardioplegic solution) for 2 min [30], and immersion stored in 0°C CP-11EB for 18 hrs.

The composition of KHB was (mM): 118 NaCl, 11 glucose, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 0.5 Na_2-EDTA, and 2.5 $CaCl_2$. The composition of CP-11EB was (mM): 111.5 NaCl, 14 KCl, 7 glucose, 10 mannitol, 15 $MgSO_4$, 1.2 KH_2PO_4, 10 Na-Hepes, 0.28 $CaCl_2$, 0.02 EDTA-Na_2, 7.5 2,3-butanedione monoxime with a pH of 7.5 (22°C), and an osmolality of 300 mOsm/kg water. Both KHB and CP-11EB solutions were filtered through 0.22 membrane filters after preparation and passed through a 3 μm inline filter during perfusion. Other than indicated, all reagents were either cell-culture-tested or American Chemical Society-grade chemicals from the Sigma Chemical Company (St. Louis, MO).

Functional assessment of the stored rat heart. After storage, the heart was reperfused in working mode with KHB [30]. Heart rate (HR, beats/min), aortic and coronary flow (AF and CF, ml/min), cardiac output (CO, the sum of AF and CF), and systolic and diastolic aortic pressure (SP and DP, mm Hg) were monitored at 5-min intervals. Coronary vascular resistance (CVR, mm Hg-min/ml) and external work (g-meter/min) were calculated [30]. Function of freshly isolated unstored heart perfused with KHB in working mode for 30 min served as the unstored control (UC).

Laser irradiation. A Lexel Aurora Argon Dye laser with wavelength ranges from 620 to 670 nM (Model 600 Dye Laser, Fremont, CA) was used for PI. Based on preliminary experiments, a wavelength of 660 nm was chosen. A power density of 40 mW/cm^2 was maintained within a 3.5 cm diameter area as measured by a Liconix laser power meter (Sunnyvale, CA). For the Pre group, the heart was irradiated in situ for 7 min (Fluence = 16.8 J/cm^2) as the anesthetized open-chest rat was artificially ventilated. For the Post group, the stored heart received PI (20 mW/cm^2) throughout the 30 min working reperfusion (Fluence = 36 J/cm^2). For the combined pre- and post-storage PI treatment (Pre/Post), the heart received laser irradiation during both periods (Fluence = 52.8 J/cm^2).

Isolated Cardiomyocyte Preparation

Cardiomyocyte isolation. The rat cardiomyocytes were isolated according to Piper et al. [31] with modifications. In brief, the rat heart was excised and perfused via an aortic cannula at a constant flow of 7.0 ml KHB-2/min for 5 min at 36.5°C. KHB-2 consists of (mM): 118 NaCl, 11.0 glucose, 10.0 Na-Hepes, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, pH adjusted to 7.5 with HCl, oxygenated with 100% O_2. The perfusion was then continued for 12 min with Enzyme-1 solution, which was KHB-2 containing 0.04% (w/v) collagenase (type II, Worthington Biochemical Corp., Freehold, NJ), 0.1% bovine serum albumin (BSA), and 50 μM $CaCl_2$. The large vessels and atria were trimmed off and the ventricles were minced into small pieces with scissors in about 10 ml Enzyme-2 solution, which was Enzyme-1 solution

containing 1% BSA. After gentle shaking at room temperature for 3 min to dislodge the dissociated cells and sedimentation of tissue fragments by gravity, the cell suspension was diluted with equal volume of Wash solution (KHB-2 containing 1% BSA and 1 mM $CaCl_2$). The remaining tissue fragments were resuspended in 10 ml of Enzyme-2 solution and the shaking and washing procedure was repeated until little of the tissue fragments remained. The viability of isolated cardiomyocytes was examined by light microscopy; only suspensions with high-percentage (>80%) of rod-shape cells were combined and washed three times by resuspension and centrifugation in the Wash solution. During isolation, all solutions and cell suspensions were saturated with 100% O_2. Finally, the isolated cardiomyocytes were resuspended in 0°C CP-11EB and stored on ice for a 20 min trip prior to laser experiments.

Warm-incubation and cold storage of the isolated cardiomyocytes. The cardiomyocytes in CP-11EB were sedimented and resuspended in 37°C medium 199 containing 10% bovine calf serum (MBS) and distributed into 24-well plates (1 ml/well) for ATP and CAT determination or 96-well plates (0.2 ml/well) for NO determination. After 1–2 hrs incubation at 37°C to allow cell attachment, most of the dead cells were removed by a change of medium. Staining with trypan blue revealed that the viability of attached cells was 85–90% at a cell density of 1–4 × 10^4/ml. The cells were either not treated or treated with laser irradiation for 4 min (660 nm, power density = 20 mW/cm^2, fluence = 4.8 J/cm^2), then either incubated in MBS at 37°C for 1 or 18 hrs or stored in CP-11EB at 4°C for 18 hrs. ATP content and CAT activity at the end of 1 hr of warm incubation, and NO release at the end of 1 and 18 hrs of 37°C incubation were determined. After 18 hrs 4°C storage, some of the wells were used for end-storage ATP determination. To simulate reperfusion, CP-11EB in other wells was replaced with 37°C MBS and the cells were further incubated at 37°C for 30 min and then assayed for end-reperfusion ATP content or CAT activity. Post-storage or combined Pre/Post storage PI treatment was not performed in the cardiomyocyte experiment.

Determination of cardiomyocyte ATP content. Cellular ATP content was determined spectrophotometrically according to the Sigma Procedure No. 366-UV. CP-11EB was removed from the wells and 1.0 ml of 12% trichloracetic acid (TCA) was added to each well to extract the metabolites. For the assay, 1 ml 3-phosphoglyceric acid-buffered solution, 1.5 ml water, 0.5 ml TCA extract, and 0.3 mg NADH were mixed and an initial absorbance at 340 nm was obtained. Then 0.04 ml of glyceraldehyde phosphate dehydrogenase/phosphoglyceric phosphokinase was added to the cuvette and a final reading at 340 nm was recorded 5 min later. The protein content was determined in each well with Pierce BCA Protein Assay method using BSA as the standard. The ATP content (nmol/mg protein) was calculated from the change in absorbance using 6.22 as the millimolar extinction coefficient of NADH.

Determination of CAT activity in the cardiomyoctes. Cells from 2 wells were combined and centrifuged at 500 rpm for 5 min. The cells were resuspended in 1 ml of 50 mM phosphate buffer (pH 7.0) and homogenized in a glass homogenizer. The suspension was then sonicated by 4 cycles of 45 W blasts (5 sec/cycle) in an ice bath. An aliquot of the homogenate (0.01 ml) was used for BCA protein assay and the rest was centrifuged at 20,000*g* for 10 min at 4°C. CAT activity in the supernatant was assayed by detecting the disappearance of hydrogen peroxide at 240 nm [32]. In brief, 0.3 ml of 20,000*g* supernatant, 0.3 ml of 50 mM phosphate buffer, and 0.3 ml of 15 mM H_2O_2 were mixed in a quartz cuvette; absorbance at zero time (A_1) and at the end of 30 sec (A_2) were recorded. Because the initial (0–30 sec) decomposition of H_2O_2 follows the first-order kinetics with H_2O_2 concentration between 10 and 50 mM, CAT activity is expressed as the reaction rate constant (k) per gram protein [32]. CAT (k/g protein) = (2.3/t)(log A_1/A_2)(a/b) = 0.23 (log A_1/A_2)/b, where t = reaction time (30 sec); $A_1$and A_2 = absorbance at 0 and 30 sec, respectively; a = dilution factor (3.0); and b = protein content (g)/0.3 ml sample.

Determination of NO release by the cardiomyocytes. NO release was quantified by measuring NO metabolite, nitrite, in the conditioned MBS [33]. Briefly, the conditioned medium was mixed with an equal volume of 1% sulfanilamide in 0.5 N HCl. Fresh MBS was used in reagent blanks. After a 5-min incubation at room temperature, an equal volume of 0.02% naphthylenediamine was added. Following an incubation at room temperature for 10 min, the absorbance at 570 nm was recorded using $NaNO_2$ as a standard. The results are expressed as μM nitrite.

Statistics

Data were expressed as mean ± SEM. Significant difference (P<0.05) between the un-

TABLE 1. Effect of 660 nm Laser Irradiation on Functional Viability of the Cold-Stored Heart†

Groups	No.	HR	AF	CF	CO	SP	DP	Work	CVR
OC	6	305 ± 23	27.5 ± 2.6	12.2 ± 1.3	39.6 ± 3.7	96 ± 4	65 ± 2	40.9 ± 5.0	5.59 ± 0.55
Pre	8	313 ± 19	33.6 ± 1.4*	13.1 ± 0.5	46.7 ± 1.7*	105 ± 2*	67 ± 2	50.5 ± 2.6*	5.15 ± 0.16
CC	8	289 ± 14	28.5 ± 1.8	11.4 ± 0.8	39.8 ± 2.6	100 ± 2	65 ± 1	41.6 ± 3.4	5.87 ± 0.41
Post	8	301 ± 14	32.8 ± 1.3**	13.2 ± 0.7	46.1 ± 1.7**	104 ± 2	67 ± 1	49.9 ± 2.5**	5.17 ± 0.3
Pre/Post	7	322 ± 12	25.3 ± 3.0	12.0 ± 1.0	37.4 ± 3.8	94 ± 4	63 ± 2	38.2 ± 5.3	5.40 ± 0.34

†The isolated rat heart was stored at 0°C for 18 hrs and reperfused at 37°C for 30 min. Laser treatment was applied either pre-storage to the heart in situ for 7 min (16.8 J/cm^2) (Pre group) or post-storage during 30 min reperfusion (Post group, 36 J/cm^2), or both (Pre/Post group, 52.8 J/cm^2). Function of the unstored control hearts was: heart rate (HR), 305 ± 14 beats/min; aortic flow (AF), 52.8 ± 1.5 ml/min; coronary flow (CF), 22.6 ± 1.3 ml/min; cardiac output (CO), 75.4 ± 2.3 ml/min; systolic pressure (SP), 129 ± 2 mmHg; diastolic pressure (DP), 78 ± 1 mmHg; work, 97.5 ± 4.3 g-m/min; coronary vascular resistance (CVR), 3.49 ± 0.21 mmHg-min/ml. OC, open-chest untreated control; Pre, pre-storage laser treatment; CC, closed-chest untreated control; Post, post-storage laser treatment; Pre/Post, laser treatment at both pre- and post-storage of the heart.
*$P < 0.05$ vs. OC group.
**$P < 0.05$ vs. CC group.

treated and the laser-treated groups was detected by unpaired t-test.

RESULTS

Hemodynamic Performance

Function of the UC hearts was listed in the legend of Table 1. After 18 hrs of storage, untreated hearts (OC and CC groups) recovered similar function with AF, CO, and work around 50% of UC (Table 1); while either the Pre or the Post group (PI treatment) recovered significantly better. AF, CO, and work was 64%, 62%, and 52% of UC in the Pre group ($P<0.05$ vs. OC group), and 62%, 61%, and 51% of UC in the Post group ($P<0.05$ vs. CC group). HR, CF, DP, and CVR did not show any statistical differences among the four groups, although there was a trend of improvement by PI treatment. Combined Pre/Post PI treatment did not show any beneficial effect compared to the OC group (Table 1).

ATP Content in the Cardiomyocytes

The ATP content in the untreated and PI-treated groups were comparable after the cardiomyocytes were incubated in MBS at 37°C for 1 hr. These values were used as prestorage controls. After 18 hrs of cold storage, the cellular ATP content decreased significantly to 48% in the untreated and 63% in the PI group relative to the corresponding control. But the end-storage ATP in the PI group was significantly higher than that of the untreated cells (Table 2), indicating prestorage PI promoted myocardial ATP preservation. After 30 min 37°C incubation to simulate reperfusion, cellular ATP rose in both untreated and PI groups to prestorage levels and there was no difference in end-reperfusion ATP between the two.

CAT Activity of the Cardiomyocytes

Prestorage CAT activity in cardiomyocytes incubated at 37°C for 1 hr did not show any difference between the untreated and PI cells (Table 2). After 18 hrs cold-storage and 30 min reperfusion, CAT activity was significantly lower than pre-storage levels in both untreated and PI cells; but the activity in the PI group was about twofold of that in the untreated cells ($P<0.05$) (Table 2).

NO Production by the Cardiomyocytes

The amount of NO released into the culture medium was similar in the untreated and PI-treated groups after 1 hr 37°C incubation. However, there was a significant increase in the NO release by the PI group ($P<0.05$ vs. untreated) after 18 hrs incubation at 37°C (Table 2).

DISCUSSION

The cardiovascular effects of PI include vasodilation in rabbit aortic rings [21,34] and hamster skin flap venules [22], increased activities of SOD and CAT in ischemic dog heart [26] and rat blood [27], and increased Ca-ATPase activity in rat cardiomyocytes [28]. Several clinical studies from Russia demonstrated that PI of the patient's blood was effective in treating angina [12,14,35] and myocardial infarction [14,15,36] by influencing circulating prostaglandins, antioxidants, adrenocortical hormones, and aldosterone-renin-angiotensin systems [14,15]. Furthermore, previous studies showed that PI enhanced serum and spleen lymphocytes NO generation in rat sepsis

TABLE 2. Effect of Laser Irradiation (660 nm, 4.8 J/cm^2) on ATP Content (nmol/mg Protein) and Catalase (CAT) Activity (*k*/g Protein) in Rat Cardiomyocytes and on Nitric Oxide (NO) Release Into the Incubation Medium (μM Nitrite)[†]

Groups	4°C Storage (hrs)	37°C Incubation (hrs)	Untreated	Laser-treated	*P*-value
ATP	—	1	64.2 ± 7.2 (7)	86.9 ± 13.0 (8)	0.0838
ATP-ES	18	—	30.7 ± 5.1 (7)	54.4 ± 7.1* (7)	0.0090
ATP-ER	18	0.5	68.6 ± 14.0 (5)	81.1 ± 21.5 (6)	0.3260
CAT	—	1	31.3 ± 2.6 (4)	32.2 ± 1.7 (8)	0.3866
CAT-ER	18	0.5	9.0 ± 0.8 (9)	17.8 ± 2.6* (9)	0.0026
NO	—	1	1.89 ± 0.11 (7)	2.14 ± 0.45 (8)	0.3099
NO	—	18	2.89 ± 0.14 (13)	3.32 ± 0.21* (13)	0.0475

[†]Samples were taken at the end of 18 hrs 4°C storage (ES), 18 hrs 4°C storage and 30 min reperfusion (ER), and 1 or 18 hrs 37°C incubation. $P<0.05$ vs. untreated group. Number in parenthesis represents assays performed.

model [29] and ATP production in normal spleen lymphocytes [25].

Because of the reported beneficial effect of PI, we tested the hypothesis that laser irradiation may improve preservation of the heart. The rat heart was treated with laser in three different settings: 1) directly on blood-perfused beating heart in situ before cold storage; 2) during post-storage reperfusion of the isolated heart with crystalloid perfusate; and 3) during both pre-storage and post-storage reperfusion periods to test any additive effect. We chose 660 nm because 630 nm, a wavelength previously found to affect lymphocytes, did not have any effect on the rat heart in preliminary experiments (data not shown). The sham-operated group (OC) served as the control for the Pre and Pre/Post groups; the CC group served as the control for the Post group. Both OC and CC hearts recovered similar function indicating that open-chest surgery and artificial ventilation did not affect preservation. Both Pre and Post groups showed significant enhancement in recovery of AF, CO, and work over the corresponding controls (Table 1). CF in the Pre and Post groups tends to increase but the differences are not significant. In the isolated working heart preparation, cardiac contractility (reflected by AF) and coronary perfusion (represented by CF) are intimately related each other, i.e., AF improvement tends to increase CF and vice versa. It is difficult to dissociate the improvment in AF from that in CF. Notwithstanding, we consider that there was an improvement of the overall cardiac function by either pre- or post-storage PI treatment.

Combined pre- and post-storage PI treatment, however, did not offer any beneficial effect (Table 1). This may be related to the dose of laser irradiation. A dose-dependent biostimulatory/inhibitory effect of PI on wound healing in rats has been reported previously [37]. Doses between 10 and 30 J/cm^2 accelerated wound healing, whereas doses higher than 30 J/cm^2 inhibited wound healing. Treating the heart with both pre- and post-storage laser irradiation would result in a total irradiation dose of 52.8 J/cm^2, which may elicit an inhibitory effect on the heart. Moreover, PI may increase the production of oxygen free radicals, which are harmful to the heart, via accelerated mitochondrial respiratory chain reactions [42]. This may be partially responsible for the inhibitory effects of the combined laser treatment.

We further investigated the possible mechanisms of the laser effect in the isolated cardiomyocyte preparation (mostly cardiomyocytes with some blood vessel cells and fibroblasts). A previous study showed that lymphocyte ATP content was significantly increased following laser treatment (630 nm) and 24 hrs of 37°C incubation [25]. We found that the ATP content was indistinguishable between untreated and laser-treated cardiomyocytes after 1 hr warm incubation. The discrepancy between the cardiomyocyte and lymphocyte might be caused by differences in wavelength, incubation periods, and the type of cells. Cardiomyocyte ATP decreased during 18 hrs of 4°C storage, but the decrease was much smaller in irradiated cells resulting in a higher end-storage ATP content in the PI group than the untreated control ($P<0.05$). Nevertheless, the difference disappeared after 30 min of reperfusion; ATP in both untreated and PI groups recovered to the prestorage level. These results suggest that the effect of PI on ATP preservation may be manifested only during cold ischemia when ATP synthesis is suppressed. Under normoxic, normothermic conditions (e.g., 1 hr of 37°C incubation or reperfusion),

PI may not have any effect on ATP level. PI was found to promote ATP synthesis in guinea pig liver mitochondria [24]. Chizhov et al. also reported that helium-neon laser irradiation causes activation of several enzymes of myocardial energy metabolism [38]. The mechanisms of increased ATP synthesis by PI may be related to the acceleration of electron transport in the respiratory chain leading to the change in the redox reactions following PI [39,40]. Thus pre-storage PI treatment might have promoted ATP synthesis in the stored heart during ischemia leading to higher end-storage ATP content which facilitated the recovery of function in these hearts.

PI has been shown to promote antioxidant enzyme activity in various experimental models. Laser (632 nm) treatment of dog heart in situ 30 min/day for 5 days enhanced coronary SOD and CAT activities by two-to-three fold [26]. Salansky et al. observed that low intensity irradiation (630 nm, 5.7 mW/cm^2, 5 min) in rat increased blood SOD and CAT level [27]. Olesin et al. reported that laser irradiation (633 and 890 nm, 3 J/cm^2) increased myocardial antioxidant system activity, reduced the Na-Ca current through the membrane, and accelerated the return of Ca^{2+} into the sarcoplasmic reticulum [28]. These effects would all favor cardiac protection by attenuating free radical injury and sodium and calcium overload during reperfusion. Furthermore, Karu et al. [41] reported that catalase activity of *T. sphaerica* increased after the He-Ne laser irradiation. To test whether PI increases CAT activity in cardiomyocytes during normoxic, normothermic incubation, CAT activity was assayed after 1 hr of 37°C incubation. No difference between the PI and the untreated groups was found. In the cold storage experiment, we assayed the end-reperfusion cardiomyocyte CAT activity, because oxygen free radicals are mostly generated during post-ischemic reperfusion. Cellular CAT activity decreased significantly from the pre-storage CAT level. But the remaining CAT activity in PI group was almost twofold of that in the untreated group, suggesting that PI retarded the decrease of rat myocyte CAT activity during cold storage. Because of the undetectable effect on rat myocyte CAT activity under normal incubation conditions, PI may only be protective during ischemia/reperfusion. Higher activity of antioxidant enzymes (e.g., CAT) in PI treated heart would make the heart less susceptible to reperfusional injury by oxygen free radials leading to better functional recovery.

Certain UV and visible lights (including laser) at low radiant doses cause vasodilation in various animal models [21–23,34,42–44]. Photovasorelaxation was considered to be endothelial-independent relaxation of the vascular smooth muscle [21,34,44]. Endothelial denudation failed to abolish PI-induced vasodilatation, neither did NO synthase inhibition by N-monomethyl-L-arginine or prostaglandin synthesis inhibition by indomethacin [21]. We observed a trend of an increase in CF and decrease in CVR in PI-treated hearts although the changes did not reach statistical significance (Table 1). Because of the previous observations of PI (630 nm, 5 J/cm^2) in promoting NO production in serum and lymphocytes of the septic rats [29], we tested whether PI has the similar effect on the isolated myocytes. Nitrite, a metabolite of NO, was assayed to represent NO production. Nitrite production in the PI group after 18 hrs of 37°C incubation was elevated relative to the untreated group. Based on Karu's hypothesis of photo-irradiation mechanism [39] and recent studies by Yu et al. [40], we postulate that photo-irradiation may accelerate mitochondrial electron transport which leads to an increase in the production of FAD, a cofactor for NO synthase. The increase in FAD production, in turn, promotes the generation of NO. Whether this observation in isolated cells is relevant to the PI effect on the intact heart is unclear, because 660 nm used in this study was beyond the active wavelength range which causes vasorelaxation [21,22,34,44].

Because we did not determine the physiologic responses of the normal unstored heart to laser irradiation, the possible contribution of these reactions to the observed effect of laser on cardiac preservation cannot be excluded until further study is performed. The differences in organ size and tissue thickness between a rat heart and a human heart is a concern; observations made on a small heart may be not applicable to a large heart. Clinical application of this observation, however, is far beyond the scope of this experiment. As to treatment of a large organ, diffuse beam fiber irradiation from both outside and inside the cardiac chamber may be a feasible approach to the problem of dealing with thick tissue of a large heart.

In summary, the present study demonstrated that either pre- or post-storage laser treatment of the rat heart significantly improved functional viability. The mechanisms for this improvement may involve better preservation of

myocardial ATP content and antioxidant enzyme activity.

REFERENCES

1. Abergel RP, Lyons RF, Castel JC, Dwyer RM, Uitto J. Biostimulation of wound healing by laser: experimental approaches in animal models and in fibroblast cultures. Dermatol Surg Oncol 1987; 13:127–133.
2. Lam TS, Abergel RP, Castel JC, Meker CA, Dwyer RM, Uitto J. Laser stimulation of collagen synthesis in human skin fibroblast culture. Laser Life Sci 1986; 1:61–77.
3. Georgadze AK, Karpov VI, Kuznetsov EV, Soldatov AV, Rukosuev VP. Treatment of non-healing wounds and trophic ulcers by low-intensity laser irradiation in an outpatient clinic. Khirurgiya 1990; 12:93–96.
4. Kuliev RA, Babaev RF, Akhmedova LM, Ragimova AI. Treatment of suppurative wounds in patients with diabetes mellitus by magnetic field and laser irradiation. Khirurgiya 1992; 7–8:30–33.
5. Kuliev RA, Babaev RF. Phase treatment of suppurative wounds using ultrasonics and laser irradiation in patients with diabetes mellitus. Klinicheskaia Khirurhiia 1992; 1:6–8.
6. Loginov AS, Sokolova GN, Trubitsyna IE, Sokolova SV, Markin EP, Varvanina GG. Biogenic amines and cyclic nucleotides in the laser therapy of long-term nonhealing stomach ulcers. Vrachebnoe Delo 1991; 1:24–27.
7. Barakaev SB. Low-intensity laser irradiation in the treatment of peptic ulcer. Klinicheskaya Meditsina 1991; 69:44–47.
8. Loginov AS, Sokolova GN, Smotrova IA, Potapova VB, Markin EP, Ambartsumian RV. Effect of laser irradiation of different intensities on the processes of healing of stomach ulcer. Sovetskaya Meditsina 1991; 2:9–11.
9. Ivaniuta OM, Dziublik AIa, Skopichenko VN. Optimal effectiveness of complex treatment of patients with chronic obstructive bronchitis by intravascular laser irradiation of blood. Problemy Tuberkuleza 1992; 5–6:21–24.
10. Demicheva EV. Laser therapy of chronic obstructive lung diseases. Sovetskaya Meditsina 1991; 2:32–36.
11. Gel'fgat EB, Samedov RI, Kurbanova ZN, Gadzhiev GG. Changes in central hemodynamics and microcirculation during laser therapy in patients with coronary insufficiency. Kardiologiia 1993; 33:22–23.
12. Vasil'ev AP, Strel'tsova NN, Shakhov GG, Nikitina VI. The effect of laser irradiation of the blood on the adenosine triphosphatase activity of the erythrocyte membranes and on the cardiac activity indices in patients with ischemic heart disease. Vrachebnoe Delo 1992; 6:61–64.
13. Korochkin IM, Kartelishev AV, Babushkina GV, Kapustina GM. Combined helium-neon laser therapy in patients with ischemic heart disease. Kardiologiya 1990; 30:24–28.
14. Korochkin IM, Barbarash OL, Chukaeva II, Kapustina GM, Klebanov GI, Berkinaev SF, Khalasekh M, Naumov OG, Teselkin IuO, Vinogradov IV. Effects of low-intensity laser irradiation on the functional activity of leukocytes and blood antioxidant system in acute myocardial infarction. Sovetskaya Meditsina 1990; 5:36–39.
15. Sirenko IuN, Savitskii SIu, Krasnitskii SS, Shabil'ianov AV, Popova LI. The use of low-intensity laser irradiation of the blood in myocardial infarction. Sovetskaya Meditsina 1990; 3:18–21.
16. Bobrov VA, Zalesskii VN. Laser correction of arrhythmia. Kardiologiya 1990; 30:76–79.
17. Fiszerman R. Treatment of non-healing wounds with 904 nm low energy laser therapy. Lasers Surg Med 1994; suppl 6:63.
18. Graham DJ, Alexander JJ. The effects of argon laser on bovine aortic endothelial and smooth muscle cell proliferation and collagen production. Curr Surg 1990; 47:27–29.
19. Abergel RP, Lam TS, Meker CA. Biostimulation of procollagen production by low energy lasers in human skin fibroblast cultures. J Invest Dermatol 1984; 82:395–402.
20. Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. Photochem Photobiol 1994; 59:167–170.
21. Chaudhry H, Lynch M, Schomacker K, Birngruber R, Gregory K, Kochevar I. Relaxation of vascular smooth muscle induced by low-power laser radiation. Photochem Photobiol 1993; 58:661–669.
22. Gourgouliatos ZF, Welch AJ, Diller KR, Aggarwal SJ. Laser-irradiation-induced relaxation of blood vessels in vivo. Lasers Surg Med 1990; 10:524–532.
23. Matsunaga K, Furchgott RF. Interactions of light and sodium nitrite in producing relaxation of rabbit aorta. J Pharmacol Exp Ther 1989; 248:687–695.
24. Morimoto Y, Arai T, Kikuchi M, Nakajima S, Nakamura H. Effect of low-intensity Argon laser irradiation on mitochondrial respiration. Lasers Surg Med 1994; 15:191–199.
25. Yu W, Naim JO, Lanzafame RJ. The effect of Photo-irradiation (laser) on lymphocyte proliferation and ATP production in vitro. FASEB J 1995; 9(3 Pt I):A239.
26. Pacholewicz JK, Ianuzzo CD, Bokhua MR, Ianuzzo SE, Daloisio C, McGrath LB. A novel method for myocardial infarction modification utilizing a helium-neon laser. Lasers Surg Med 1994; suppl 6:65.
27. Salansky N, Brill G, Filonenko N. Blood indexes changes under low energy photon (LEP) irradiation in vivo experiments. Lasers Surg Med 1994; suppl 6:9.
28. Olesin AI, Maksimov VA, Mazhara IuP, Pavlova RN, Lobanov NA, D'iachuk GI. Biological effect of laser radiation on the functional state of cardiomyocytes. Patologicheskaia Fiziologiia Eksperimentalnaia Terapiia 1992; 5–6:17–20.
29. Chi LH, Yu W, Naim JO, Lanzafame RJ. Increased synthesis of nitric oxide by laser irradiation in sepsis. Lasers Surg Med 1995; supp. 7:19.
30. Zhu Q, Yang X, Claydon MA, Hicks GL, Wang T. Adenosine deaminase inhibitor in cardioplegia enhanced function preservation of the hypothermically stored rat heart. Transplantation 1994; 57:35–40.
31. Piper HM, Volz A, Schwartz P. Adult ventricular rat heart muscle cells. In: Piper HM, ed. "Cell Culture Techniques in Heart and Vessel Research." Berlin, Heidelberg: Springer-Verlag, 1990, pp 36–42.
32. Aebi H. Catalase in vitro. In: Packer L, ed. "Methods in Enzymology." Orlando: Academic Press, 1984; 105:121–126.
33. Green SJ. Measuring nitrite in tissue culture supernatant. In: Coligan JE, Kruisbeek AM, Margulies DH, She-

vach EM, and Strober W, ed. "Current Protocols in Immunology." New York: John Wiley and Sons, 1994, pp 14.5.1–14.5.3.
34. Steg PG, Gal D, Rongione AJ, DeJesus SR, Clark RH, Levine HJ, Isner JM. Effect of argon laser irradiation on rabbit aortic smooth muscle: evidence for endothelium independent contraction and relaxation. Cardiovas Res 1988; 22:747–753.
35. Zubkova SM, Sorokina EI, Kenevich NA, Tupitsyna IuIu, Minenkov AA. The blood antioxidant system in patients with ischemic heart disease undergoing laser therapy. Vopr Kurortol Fizioter Lech Fiz Kult 1993; 6:4–7.
36. Kipshidze NN, Chapidze GE, Bokhua MR, Marsagishvili LA. Effectiveness of blood irradiation using a helium-neon laser in the acute period of myocardial infarction. Sov Med 1990; 3:9–12.
37. Al-Watban FAH, Zhang XY. Laser biostimulator and bioinhibitor. Lasers Surg Med 1994; suppl 6:9.
38. Chizhov GK, Koval'skaia NI, Kozlov VI. The effect of helium-neon laser radiation on the energy metabolic indices of the myocardium. Biulleten Eksperimentalnoi Biologii Meditsiny 1991; 111:302–305.
39. Karu TI. Molecular mechanism of the therapeutic effect of low-intensity laser radiation. Laser Life Sci 1988; 2:53–74.
40. Yu W, Neim JO, Lanzafame RJ. Mechanism of low level laser biostimulatory effect. Laser Surg Med 1996; suppl 8:7.
41. Karu TI, Lyapunova TS, Pomoshnikova NA. The activation of yeast metabolism with He-Ne laser radiation. IV. Relationship between the activity of catalase and protein synthesis. Laser Life Sci 1993; 5:251–258.
42. Mosseri M, Gotsman MS, Isner JM. Laser-tissue interaction: vascular reactivity. Isr J Med Sci 1993; 29:812–816.
43. Schwengel RH, Gregory KW, Hearne SE, Scott HJ, Beauman GJ, Mergner WJ, Caplin JL, Ziskind AA. Characterization of pulsed-dye laser-mediated vasodilation in a rabbit femoral artery model of vasoconstriction. Lasers Surg Med 1993; 13:284–295.
44. Steg PG, Rongione AJ, DeJesus ST, Clark RH, Isner JM. Pulsed ultraviolet laser irradiation produces endothelium-independent relaxation of vascular smooth muscle. Circulation 1989; 80:189–197.